Molecular photoswitches mediating the strain-driven disassembly of supramolecular tubules

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Edited by Thomas E. Mallouk, The Pennsylvania State University, University Park, PA, and approved September 12, 2017 (received for review June 21, 2017)

Chemists have created molecular machines and switches with specific mechanical responses that were typically demonstrated in solution, where mechanically relevant motion is dissipated in the Brownian storm. The next challenge consists of designing specific mechanisms through which the action of individual molecules is transmitted to a supramolecular architecture, with a sense of directionality. Cellular microtubules are capable of meeting such a challenge. While their capacity to generate pushing forces by ratcheting growth is well known, conversely these versatile machines can also pull microscopic objects apart through a burst of their rigid tubular structure. One essential feature of this disassembling mechanism is the accumulation of strain in the tubules, which develops when tubulin dimers change shape, triggered by a hydrolysis event. We envision a strategy toward supramolecular machines generating directional pulling forces by harnessing the mechanically purposeful motion of molecular switches in supramolecular tubules. Here, we report on wholly synthetic, water-soluble, and chiral tubules that incorporate photoswitchable building blocks in their supramolecular architecture. Under illumination, these tubules display a nonlinear operation mode, by which light is transformed into units of strain by the shape changes of individual switches, until a threshold is reached and the tubules unleash the strain energy. The operation of this wholly synthetic and stripped-down system compares to the conformational wave by which cellular microtubules disassemble. Additionally, atomistic simulations provide molecular insight into how strain accumulates to induce destabilization. Our findings pave the way toward supramolecular machines that would photogenerate pulling forces, at the nanoscale and beyond.

artificial molecular switches | supramolecular polymers | supramolecular machines | light

Essentially all motion in living organisms emerges from the collective action of molecular machines transforming chemical energy into ordered activity. Inspired by nature’s machinery, chemists have moved from building static molecular structures to designing and synthesizing molecules that display mechanically relevant motion (1, 2), such as molecular switches (3, 4), pincers (5), motors (6–8), pumps (9), shuttles (9, 10), muscles (11), walkers (12), robotic arms (13), artificial peptide synthesizers (14), and self-propelled molecular cars exhibiting directional motion (15). The operation of these small molecules has been studied in solution primarily, where any mechanical action is overwhelmed by random Brownian motion.

Remarkably, nature’s molecular machines also operate in a liquid environment, where despite the Brownian storm and the constant flux of building blocks they generate strong directional forces (16) and synthesize essential molecules (17). Two characteristics underpin evolutionary designs: first, nonequilibrium operation of supramolecular machines is maintained by constant influx of chemical fuels and second, these machines are integrated into even larger supramolecular assemblies such as filaments, membranes, or tissues, to guide and coordinate the overall operation against the Brownian storm.

Reaching this remarkable level of functionality in artificial systems requires strategies where mechanically purposeful molecular motion can be transmitted effectively into motion at the supramolecular level— a challenge that has thus far proven elusive.

Cellular microtubules are versatile supramolecular machines that are capable of producing two types of directional forces under continuous influx of energy: they pull chromosomes apart through catastrophic disassembly, and shape-shift cells as they grow using chemical energy (18). This capacity to produce forces efficiently is inherently encoded into their supramolecular architecture: microtubules are in essence stiff cylinders that are self-assembled from molecules that undergo chemically fueled conformational switching between assembling and nonassembling forms (19). Although the functional disassembly of cellular microtubules is still not understood fully, it appears that conformational changes in the tubules’ building blocks induce a strain that builds up and releases abruptly to produce a directional mechanical force upon disassembly (20), a process that has been described theoretically by the conformational wave model (21, 22).

Here, we report the creation of synthetic supramolecular tubules in which the structural change at the level of individual building blocks is controlled by photochromic switching, which

Significance

Developing molecular machines has been a leading goal for scientists, but to be practically valuable their mechanically relevant motion must be decoupled from the Brownian storm that dominates in solution. The disassembly of cellular tubules operates by the switching of the shapes of the building blocks, ultimately pulling sets of chromosomes apart. Here, we show that artificial photoswitches can be incorporated within supramolecular microtubules and that individual switching events lead to conversion of light into elastic energy that can be stored, accumulated, and subsequently released to produce a mechanical effect. The work paves the way toward fully artificial supramolecular machines that convert molecular motion into sophisticated operation modes, at length scales that are typically the realm of living matter.

Author contributions: J.W.F., A.M.-A., and S.K. performed research; B.M. contributed new reagents/analytic tools; J.W.F., A.M.-A., S.K., D.B., M.C.A.S., J.H., N.K., and T.K. analyzed data; D.B. performed the simulations; M.C.A.S. performed the cryo-TEM experiments; G.M.P. designed and supervised the modeling work; T.K. designed and supervised the research; and N.K., G.M.P., and T.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

See Commentary on page 11804.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1711184114/-/DCSupplemental.
Results and Discussion

Molecular Design. The use of water as a solvent requires the engineering of photosensitive building blocks that spontaneously self-assemble in water. Our design involves a V-shaped aromatic core in which two azobenzene photoswitches are incorporated as shape-changing activators (Fig. 1B). The V-shaped core is connected to branched hydrophilic oligoether chains that help solubilize the molecules in water and imbues them with an amphiphilic character. In the absence of UV light, the planar trans form is present, and the self-assembly of the building blocks into tubes is thus expected to be driven by the combination of hydrophobic effects, \( \pi-\pi \) interactions and shape recognition, as has been reported for other shape-persistent building blocks (23, 24, 34). In contrast, the cis form disrupts both planarity and the V shape of the hydrophobic part and our design thus builds on the concept that trans–cis isomerization should promote switching between the assembling and nonassembling forms of the building blocks (Fig. 1).

Self-Assembly of Synthetic Tubules in Water. In water, both building blocks 1 and 2 form noncovalent hexameric macrocycles that stack on top of each other to form tubules (Fig. L4), as manifested in the elongated architectures observed in the transmission electron micrographs (Fig. 2A for 1, and SI Appendix, Fig. S7C for 2). Atomic

contributing to pushing the system gradually toward higher energy states, and eventually induces its catastrophic disassembly (Fig. L4). Light constitutes an ideal source of energy that can be supplied continuously to an isolated system to obtain structural switching—as opposed to previously employed external environmental triggers that do not modify the structure of the building blocks [i.e., salinity (23) and temperature (24)]. Notwithstanding the tubular shape, a number of pioneering reports on supramolecular fibers (25, 26) have demonstrated strategies to achieve out-of-equilibrium operation (27–30). The mechanism of assembly of supramolecular polymers, mostly fiber-like objects, has attracted much attention (31, 32), but much less is known about the mechanism by which these fibers disassemble (29).

In addition to the presence of a hollow cavity core that is intrinsic to a tubule (33), a major difference between supramolecular tubules and fibers lies in the rigidity of the tubules versus the flexibility of the fibers. Consequently, from a dynamic point of view, synthetic tubules can be expected to exhibit a much slower exchange rate between building blocks in solution and building blocks in the tubule, which also substantially impacts their disassembly mechanism. Herein we describe how these molecular building blocks can be encoded into a complex supramolecular system that operates in water and demonstrate that this strategy results in a nonlinear, three-step mechanism that mimics the conformational wave disassembly of cellular microtubules (22).

Results and Discussion

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mixing a 1:1 ratio of preformed chiral tubules from 1 (2.5 μM) and preformed achiral tubules from 2 (2.5 μM) and compare the dynamic behavior in either pure water (black line) or in water/acetonitrile 95/5 (red line). Over 24 h, chiral amplification is apparent in water/acetonitrile and is translated into a gradual increase of CD signal, which indicates that the chiral and achiral building blocks are mixing to form a larger number of chiral tubes (i.e., the system displays a sergeants and soldiers effect). In stark contrast, chiral amplification is not observed in water. The absence of chiral amplification indicates that in water exchange between the building blocks, which the tubules are composed of, does not occur. These data indicate that in water and over the timescale of the measurements, the structure of the tubules is kinetically trapped at room temperature. (B) CD signal of 1 recorded at λ = 390 nm in water (2.5 μM), for varying temperatures.

force microscopy (AFM) also confirms these findings (SI Appendix, Fig. S8). Cryo-TEM images reveal the internal aromatic part of the tubules with the uniform size along its whole length (Fig. 2B). The measured diameter (d ≈ 5 nm) corresponds to the expected diameter of the hexamer (SI Appendix, Fig. S9). The periodicity of bundled tubules indicates the external diameter of the tubule is ca. 11 nm, in agreement with the value estimated from molecular models (SI Appendix, Figs. S9 and S10). TEM micrographs indicate an external diameter of ca. 7 nm for both 1 and 2 (SI Appendix, Fig. S7), which we attribute to a difference in sample preparation, when out of solution the hydrophilic chains interdigitate partially upon drying.

The structural information provided by microscopy is complemented by spectroscopic data. Upon self-assembly of 1 in water, a CD signal appears with a zero-crossing point that corresponds to the λ_{max} of the azobenzene chromophores. The intensity of the CD signal increases with the concentration of 1, indicating that 1 assembles into tubules with a preferred handedness (Fig. 2C). The shape of the CD spectrum remains constant between 1 μM and 83 μM, which indicates that the supramolecular structures are robust and uniform. A CD signal is not observed over the same concentration range when 2 is dissolved in acetonitrile, which indicates that molecular tubes do not form in acetonitrile. Achiral building block 2 does not yield a CD signal in water, which we attribute to the formation of tubules without preferential handedness.

Effective control over the dynamics of the synthetic tubules was achieved close to the critical aggregation concentration, where small changes in concentration can drive substantial changes at the supramolecular level. The critical aggregation concentration was determined by plotting changes in the fluorescence of a probe (Nile red) that favors hydrophobic cavities (35), for increasing concentrations of building blocks, and was found to be ~1 μM (Fig. 2D and SI Appendix, Fig. S11).

Synthetic supramolecular architectures are dynamic in nature and, to date, their design has been such that they readily adapt to changing boundary conditions. As soon as the conditions change, the rapid exchange between the assembled building blocks and the isolated building blocks secures a fast reequilibration process. In contrast, we anticipated that if the exchange between the assembled building blocks and those in solution is slowed, then the architectures will display a tendency to linger out of equilibrium, in a higher energy state. Nature’s supramolecular machines have evolved in such a way that they can operate out of equilibrium and employ this higher energy state to perform useful tasks. To examine the exchange rate between building blocks forming the tubules and those in solution, we mixed two populations of the individually preformed tubules formed by chiral 1 and achiral 2. Upon mixing an aqueous solution of the chiral tubules 1 with another aqueous solution containing tubules 2 the CD signal remained constant for 24 h (Fig. 3A). In contrast, upon mixing the same tubules in water/acetonitrile (95/5) the CD signal increased and revealed amplification of chirality, an effect known as the sergeants and soldiers principle (36). These data indicate that in water the exchange of molecules between the tubules and the solution is slow. In contrast, this exchange is fast in the water/acetonitrile mixture, likely because of the higher solubility of all building blocks in acetonitrile.

Further insight into the equilibrium dynamics of the tubules formed by 1 can be extracted from the temperature dependence of the CD spectra. Overall, the intensity of the CD signal recorded at λ = 390 nm decreases with an increasing temperature, until it completely disappears at 55 °C (Fig. 3B). The disappearance of the CD signal most likely originates from the greater thermal energy that decreases the average length of the tubules until they become soluble. While at temperatures between 40 °C and 55 °C the CD signal decreases linearly, below...
At lower concentrations of 1 in water (2.5 μM), full disassembly is achieved (Fig. 5A). Parallel monitoring of the disassembly process by CD and by UV-visible (UV-vis) absorption spectroscopy shows that the complete disassembly is achieved after 35 min of irradiation, that is, before a photostationary state is reached (Fig. 5B), with the trans/cis ratio reaching 83/17 only (SI Appendix, Fig. S15). The photo-triggered decrease in the CD signal does not follow a monoeponential behavior (Figs. 5A and 6A). Instead, 80% of the CD signal is lost abruptly within the first minutes of irradiation, when only 7% of the trans form has switched into the cis form. We attribute this first phase to a reduction of the helical twist, which is accompanied by a red shift of the wavelength of maximum absorbance λ_max (Figs. 5B and 6B and SI Appendix, Fig. S16). Such a red shift indicates a change toward another molecular organization (J-aggregates) (37), leading us to conclude that the building blocks slide with respect to each other, within the tubules. After 5 min, when the trans/cis ratio is estimated at 93/7, the λ_max reaches its maximum value and remains constant for a further 30 min of irradiation (Figs. 5B and 6B). During this time, the CD signal continues to decrease, albeit at a slower rate. After 35 min of irradiation, the λ_max starts to shift

Fig. 5. Conversion of light into molecular and supramolecular strain. (A) Evolution of the CD spectra of tubules formed by 1 (2.5 μM solution of 1 in water) as a function of the time upon irradiation (measured at 0, 1, 2, 5, 10, 20, 35, 50, and 60 min) with UV light (λ = 365 nm). The disappearance of the CD signal after 35 min indicates that the tubules have disappeared. (B) Absorption spectral changes induced by irradiation with UV light.

40 °C the system starts exhibiting a nonlinear behavior. Between 25 °C and 35 °C the intensity of the CD signal decreases by less than by 10%, whereas between 35 °C and 40 °C the CD signal abruptly reduces by 50%. This shows that the assembled tubules are kinetically trapped around room temperature within the timescale of the measurements, whereas at elevated temperatures the structure dynamically adapts to temperature changes. Although the response of the self-assembled tubules to temperature cannot be directly compared with the situation when the system responses to structural changes of the building blocks, it gives a valuable insight into the dynamics of the molecular exchange between the assembled and free state. Overall, we conclude that at ambient temperature the system of tubules in water (2.5 μM) at λ_max position of 367 nm and a less pronounced increase of the n–π* absorption around 450 nm.

Illumination of the tubules (2, 1.33 mM) for less than 20 min did not yield a significant change in either their length (Fig. 4A and B) or diameter (increased by ~1 nm, SI Appendix, Fig. S12). The marginal increase in length falls within the experimental uncertainty. Further, there are always 1 μM of nonassembled building blocks in solution (as indicated by the value of the critical aggregation concentration before irradiation), but their low number cannot account for this marginal length increase at a concentration of 1.33 mM.

After 60 min of irradiation, cryo-TEM images show that the tubules become shorter (Fig. 4C) and that their number increases (SI Appendix, Fig. S13). The distribution in length shows the presence of shorter tubules after 60 min of UV irradiation with the disappearance of long tubules (>300 nm) compared with the distribution before irradiation or after irradiation for 20 min (Fig. 4D–F).

Combined, these observations indicate that the tubules break into shorter segments, tentatively at positions where the azobenzene groups are switched to the cis form. Dynamic light scattering (DLS) data obtained in situ, at lower concentrations, support this conclusion further (100 μM, SI Appendix, Fig. S14). At concentrations of ~1.33 mM used in the cryo-TEM measurements, complete disassembly of the tubules was not observed, as the remaining concentration of the trans form at the photostationary state is considerably greater than the critical aggregation concentration.
toward shorter wavelengths, while the CD signal approaches zero. Once irradiation stops, the building blocks undergo thermal cis-to-trans switching (SI Appendix, Fig. S6); however, full reversibility of the supramolecular system is not achieved in these experimental conditions, likely because constant recovery of the trans form does not mediate the same cooperative effects as in a situation that involves the trans form exclusively.

When Nile red (0.94 μM) is encapsulated in the tubules formed by 1 in water (2.5 μM), the changes in UV-vis absorbance and CD spectra upon irradiation are the same as in the absence of the fluorescent probe (SI Appendix, Fig. S17), which indicates that the encapsulation of the Nile red does not alter the phototriggered disassembly pathway. Release of Nile red expected to occur alongside the disassembly of the tubules reflects the complex disassembly process. The release of Nile red is manifested in changes in fluorescence. During the first minutes of irradiation the intensity of the fluorescent signal increases (Fig. 6), which indicates that Nile red is released and the tubules are disassembling.

Insights into the Mechanical Operation of the Tubules. Overall, irradiation of the self-assembled tubules pushes them out of equilibrium and toward a complex stepwise disassembly (Fig. 6). First, when irradiation begins, the trans form of the azobenzene switches to the cis form at numbers that remain sufficiently low to be stabilized within the tubules, without forcing them to disassemble. The strain induced by the nonplanar and bended cis form is balanced by the free energy that would be required to solubilize the cis form in water. As irradiation proceeds, the increasing amount of cis form gradually builds up further strain. Next, a critical amount of cis form within the tubules is exceeded and they start to break up, most likely at places where the concentration of the cis form is the highest in the tubules. The breaking of the tubules decreases the effective length of the hydrophobic cavity where the fluorescent probe can be encapsulated, which results in its gradual release and decrease of fluorescence (Fig. 6C). Finally, when the tubules become shorter than their critical nucleation size they disappear completely. This final phase of the disassembly comes with a clear blue shift of the λmax, as a sizeable fraction of the molecules are in the cis form and consequently start dispersing from the assembled state into individually solubilized molecules. The absence of isodichroic point is in agreement with switching from a CD-active assembly to a CD-inactive solution of molecules.

Our results also show that the exchange rate of the building blocks has a significant impact on the dynamics of the system. Irradiation of the tubules in a water/acetonitrile (95/5) solution is not accompanied by a shift of the absorption band. The CD signal consistently shows a simple behavior (SI Appendix, Fig. S18), which indicates that in the presence of an organic solvent the disassembly loses its complexity and follows a simple process, where the initial lag phase and buildup of strain are typically absent.

Alternative mechanisms for the dynamic molecular behavior on which we report can be excluded on the basis that they fail to support experimental evidence, including (i) the possibility of slow dissolution of the cis form and subsequent reequilibration of the assemblies that would be dictated by a lower concentration of the trans form in the system and (ii) the hypothesis that trans-to-cis isomerization would occur at a higher rate at the edge of the tubules, and induce dissolution of the cis form. In both cases, the tubules would undoubtedly shorten rather than break and, moreover, fast reequilibration within the timescale of minutes would be required, which clearly does not happen in the current system (Fig. 3). Furthermore, these alternative mechanisms would not account for the observed spectral shifts, nor would the change of solvent alter the observed behavior.

Strain Buildup and Tubule Destabilization Captured by All-Atom Simulations. Atomistic modeling provides further insight into the strain buildup in the tubules. Atomistic models for 1 and for the corresponding equilibrated tubule are shown in Fig. 7 (computational details are provided in SI Appendix). Tubule 1 was equilibrated and found very stable during 150 ns of an all-atom molecular dynamics (AA-MD) simulation (SI Appendix, Fig. S19). As used recently in the study of molecular transitions in supramolecular polymers (38), starting from equilibrated tubule 1 (Fig. 7A) we used well-tempered metadynamics (39) to calculate the minimum energy that is necessary for trans-to-cis isomerization in a monomer within the tubule (SI Appendix, Fig. S20). We used this information to set up out-of-equilibrium AA-MD simulations where trans-to-cis isomerization could be observed iteratively. The C=N=C dihedral angle potential of the assembled monomers was modified to disfavor the trans form (SI Appendix), and as a result switching to the cis form was induced in the course of the simulation. This condition is consistent with all monomers being uniformly irradiated by light, while each transition depends on the crowding around individual monomers.

Fig. 7. Strain build-up and tubule destabilization captured by all-atom simulations. (A) Atomistic models of building block 1 and of the unperturbed original tubule. (B) Energy absorbed by the tubule (ΔE, values per-monomer) as a function of the percentage of cis form. (C) Deviation from the ordered arrangement of the monomers in unperturbed 1 (ΔΦ, in percentage), as a function of the percentage of cis form. Data are averaged data from three replica simulations. (D) Snapshots of tubule from 1 at the start (0%) and once trans-to-cis isomerization has reached 20%. Aromatic units are colored gray, internal water molecules are colored red while external water molecules are blue (PEG groups are not shown for clarity; they appear as voids). Above 20% of trans-to-cis isomerization, water diffuses in and out of holes in the structure (black circle).
During simulations, the azobenzenes were seen to undergo trans-to-cis isomerization, which allows monitoring of the energy $\Delta E$ absorbed by the assembly, as a function of the increasing percentage of cis form in the tubule (Fig. 7B). $\Delta E$ correlates to the strain energy that builds up in the system. The results demonstrate that the accumulation of strain in a tubule is nonlinear. In particular, while below $\sim 10\%$ of trans-cis transition we observe an initial phase where the supramolecular structure can keep the $\Delta E$ constant ($\Delta E \sim 2 \text{ kcal mol}^{-1}$, average value per monomer in the system), above $\sim 15\%$ the $\Delta E$ rises considerably.

Analysis of how much the monomer arrangement in the assembly deviates from the ordered one in the original tubule (Fig. 7C, $\Delta \Phi$) shows that structural deformations in the tube are also nonlinear. Globally, the system shows a “stop-and-go” behavior, where the tubule accumulates energy, which is then released as plastic deformations of the structure. In fact, above $\sim 15\%$ of trans-cis isomerization we clearly observe the appearance of holes in the tubule (Fig. 7D), which constitutes further evidence of structural collapse.

These simulations are representative of a reduced portion of an infinite supramolecular tubule and are limited to a 100-ns timescale. However, while not allowing us to observe full disassembly of the tubules into monomers during the runs, the model provides evidence of the structural and energetic impairment introduced into the assembly by increasing levels of trans-to-cis isomerization, which are the key molecular factors underpinning tubule disassembly by exposure to light.

**Conclusion.** A complex, multistep, light-driven disassembly of aqueous supramolecular tubular assemblies has been demonstrated. The initial conversion of light into tubular strain, which is stored and accumulated before the system breaks apart, is reminiscent of the strain-driven disassembly of cellular microtubules, by which these biological machines generate forces that pull chromosomes apart—another challenge is to combine this operation mode with their complementary operation mechanism, where they push objects by ratcheting upon growing. Here, the strain energy that accumulates during the initial phase is related to the number of azobenzene units present in the cis form and the difference between the free energy gained by insertion of either the trans form (negative) or the cis form (positive) to the tubular structure. Experimental quantification of this strain energy and its use to produce directional work is ongoing. Ultimately, this system shows potential toward transducing the mechanical action of photoswitches across length scales, as in the process of vision, where large geometrical changes associated with double-bond isomerization are phototransduced by supramolecular assemblies, to yield macroscopic phenomena. We envision that in the future such synthetic molecular self-assembled architectures will be capable of generating directional forces at the nanoscale (e.g., by deformation of vesicular walls).

**Materials and Methods**

Details of materials, instruments, and methods are provided in SI Appendix, including synthesis and characterization of the compounds, optical spectroscopy measurements, DLS, TEM, cryo-TEM, AFM, determination of the critical aggregation concentration, and computational calculation methods. Measurement of length and diameter of the tubules in AFM, TEM, and cryo-TEM images was done using ImageJ 1.46 software.

**ACKNOWLEDGMENTS.** This work was supported by Netherlands Organization for Scientific Research NWO Project 726.011.001 and European Research Council Starting Grant Phelix (to N.K.). D.B. and G.M.P. acknowledge Swiss National Science Foundation Grant 200011_162827 (to G.M.P.).

Supporting Information

Molecular photo-switches mediating the strain-driven disassembly of supramolecular tubules

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1. General methods

**Materials.** Reagents and solvents were purchased from commercial sources and used without further purification unless stated otherwise. $^1$H and $^{13}$C NMR were recorded at 400 and 100.6 MHz, respectively. 2D COSY and HMQC experiments were used to assist on NMR peak assignments. Chemical shifts are reported in $\delta =$ units (ppm) relative to the residual protonated solvent signals of CDCl$_3$ ($^1$H NMR: $\delta = 7.26$ ppm) and CD$_3$CN ($^1$H NMR: $\delta = 1.94$ ppm). Thin-layer chromatography (TLC) was carried out using Merck silica gel 60 on aluminum sheet, with visualization by UV light and by charring with a potassium permanganate solution (2%) and sodium hydrogen carbonate (4%) in water. Column chromatography was carried out using Merck silica gel 60 (230-400 Mesh). ESI-MS spectra were obtained for samples dissolved in DCM-MeOH at low μM concentrations.

**UV-visible and circular dichroism (CD) spectroscopy.** The solutions were prepared with concentrations in the micromolar range, at least 18 hours before the irradiation experiments, from a concentrated stock solution in water. All solutions were kept in the dark at all times. UV-visible spectra were recorded at room temperature with a Perkin Elmer Lambda 850 UV-visible spectrometer in 1-cm quartz cells. CD spectra were recorded with a Jasco J-1500 spectrometer. For experiments in water/acetonitrile, acetonitrile was added to a solution of the molecules in water, before irradiation.

**Determination of critical aggregation concentrations.** Critical aggregation concentrations were determined following a reported procedure (1). Briefly, Nile Red at a concentration of 0.94 μM in milliQ water was used as a fluorescent probe. Dilutions of 1 and 2 were prepared in dye solution and incubated at room temperature for at least 15 min. Fluorescence emission spectra between 580-720 nm were recorded using a fluorescence spectrophotometer (Perkin Elmer LS55) using an excitation at $\lambda = 550$ nm.

**Dynamic Light Scattering.** The samples were prepared a day prior to the acquisition. UV irradiated samples were measured immediately after the irradiation has stopped. DLS measurements were carried out using a Nanotrac (Anaspec) particle analyzer. For each condition, five measurements of 120 s were performed, with a set-zero time of 180 s. Data was analyzed assuming a refractivity index of 1.45. Measurements were carried out at a concentration 80 μM. Particle size was determined following the intensity distribution.

**Cryo-TEM.** The solutions were prepared by solubilizing the compounds in deionized water at 1.33 mM, one day before the acquisition of micrographs. These solutions were used directly after irradiation. The cryo-TEM samples were prepared by depositing a few microliters of these solutions on carbon-coated grids (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After blotting the excess liquid, the grids were vitrified in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands) and transferred to a Philips CM 120 microscope equipped with a Gatan model 626 cryo-stage operating at 120 kV. Micrographs were recorded under low-dose conditions with a slow-scan CCD camera. The highest density is obtained from the electron dense aromatic part of the building blocks.

**Transmission electron microscopy (TEM).** The samples were prepared by drop-casting 80 μM water solutions on carbon grids (Formvar/Carbon 200 mesh, Copper). Prior to measurements the samples were stained by Uranyl acetate. TEM micrographs were recorded using a Philips CM300ST - FEG microscope.

**Atomic force microscopy (AFM).** The samples were prepared by drop casting a solution of tubules in water, on freshly cleaved mica. After blotting the excess liquid, the samples were dried in air several hours
and characterized by in tapping mode (Nanoscope IV). The AFM images were analyzed with the LA 1730 software.

**Measurements of the diameter and length of the tubules.** The Image J 1.46 software was used to analyze AFM images and (cryo-)TEM micrographs. Manual analysis led to determination of the length and diameter of the tubules. Longer tubules are underrepresented as their length was measured only within the size of the micrographs at the suitable magnification.

**Irradiation experiments.** The samples were irradiated by using a bluepoint LED Hoenle Technology lamp (300 mW cm^-2, \( \lambda = 365 \text{ nm} \)).

### 2. Synthesis and characterization

Compounds 1 and 2 were synthesized according to a **Scheme 1**. Compounds 3 (2), 6 (3) and 7 (4) were synthesized according to reported procedures.

**Scheme 1. Synthetic route towards 1 and 2.**

\((E)-4'-(4\text{-iodophenyl})\text{diazienyl})-[1,1'\text{-biphenyl}]4\text{-carbonitrile (4).} 4'\text{-Aminobiphenyl-4-carbonitrile (87 mg, 0.45 mmol, 1 eq.) and 3 (105 mg, 0.45 mmol) were dissolved in acetic acid-EtOAc 1:1 (4 mL). The reaction mixture was stirred at 40 °C and an orange solid was formed after 20 min. The reaction was stirred}
for 5 h. The precipitated compound was filtered and washed with water, then re-dissolved in dichloromethane, dried over Na$_2$SO$_4$, and concentrated to give 4. Yield: 140 mg (70%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.03 (d, $J = 8.6$ Hz, 2H), 7.89 (d, $J = 8.7$ Hz, 2H), 7.77 – 7.73 (m, 6H), 7.68 (d, $J = 8.7$ Hz, 2H). $^{13}$C NMR (100.3 MHz, CDCl$_3$) $\delta$ 152.5, 152.1, 144.7, 141.9, 138.6, 132.9, 128.0, 124.7, 123.9, 118.9, 111.7, 98.3. ESI MS ($m/z$) calculated for [C$_{19}$H$_{12}$IN$_3$H]$^+$ 410.02, found: 410.29.

($E$)-4'-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazenyl)-[1,1'-biphenyl]-4-carbonitrile (5). A solution of 4 (150 mg, 0.12 mmol), KOAc (35.3 mg, 0.36 mmol, 3 eq.), bis(pinacolato)diboron (138 mg, 0.18 mmol, 1.5 eq.) and a catalytic amount of Pd(dppf)Cl$_2$ (3.5 mg) in dry and degassed dioxane (3 mL) was stirred at 80 °C overnight. The solution was cooled down, diluted with dichloromethane and washed with water, then dried over MgSO$_4$, filtered and concentrated. The residue was purified by chromatography in toluene-hexane 9:1 → toluene-MeOH 99:1 to give 5 as an orange solid. Yield: 75 mg (50%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.04 (d, $J = 8.6$ Hz, 2H), 7.98 (d, $J = 8.4$ Hz, 2H), 7.92 (d, $J = 8.4$ Hz, 2H), 7.76 (m, 6H), 1.38 (s, 12H). $^{13}$C NMR (100.3 MHz, CDCl$_3$) $\delta$ 154.5, 152.7, 144.7, 141.7, 135.8, 132.9, 128.2, 128.0, 123.9, 122.2, 118.9, 111.6, 84.3, 25.1. ESI MS ($m/z$) calculated for [C$_{25}$H$_{24}$BN$_3$O$_2$H]$^+$ 410.20, found: 410.08.

Compound 1 and 2. A solution of 5 (60 mg, 0.15 mmol, 3 eq.), 6 (or 7) (54 mg, 0.05 mmol), Pd(PPh$_3$)$_4$ (6 mg) and aqueous Na$_2$CO$_3$ (2 M, 5 mL) in THF (8 mL) was degassed and stirred under N$_2$ atmosphere at 80 °C for 48 h. The layers were separated and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with water, dried (MgSO$_4$), filtered and concentrated. The residue was purified by chromatography in EtOAc-MeOH 24:1→ 23:2 to yield compound 1 (or 2) as an orange solid. Yield: 56 mg (74%) for 1 and 47 mg (62%) for 2.

(1) $^1$H NMR (400 MHz, CD$_3$CN) $\delta$ 7.96 (dd, $J = 8.6$, 1.8 Hz, 8H), 7.90 – 7.76 (m, 16H), 7.55 (s, 1H), 7.25 (d, $J = 1.4$ Hz, 2H), 4.19 (d, $J = 5.7$ Hz, 2H), 3.60 – 3.27 (m, 64H), 3.24 (s, 12H), 2.36 (dt, $J = 11.9$, 5.9 Hz, 1H), 1.00 (d, $J = 6.3$ Hz, 12H). $^{13}$C NMR (100.3 MHz, CD$_3$CN) $\delta$ 161.1, 153.3, 152.8, 142.7, 142.3, 133.8, 129.1, 129.0, 128.7, 124.3, 124.3, 114.0, 112.2, 75.6, 75.5, 72.6, 71.4, 71.1, 71.0, 70.2, 69.9, 68.0, 58.9, 42.0, 17.4. HR-MS ($m/z$) calculated for [C$_{88}$H$_{116}$N$_6$O$_{19}$H]$^+$ 1560.8295, found 1560.8214.

(2) $^1$H NMR (400 MHz, CD$_3$CN) $\delta$ 8.03 (dd, $J = 8.6$, 1.6 Hz, 8H), 7.94 (d, $J = 8.6$ Hz, 4H), 7.90 – 7.83 (m, 12H), 7.63 (t, $J = 1.4$ Hz, 1H), 7.32 (d, $J = 1.4$ Hz, 2H), 4.23 (d, $J = 5.6$ Hz, 2H), 3.58 – 3.54 (m, 4H), 3.51 – 3.40 (m, 64H), 3.25 (s, 12H), 2.38 (dt, $J = 11.9$, 5.9 Hz, 1H), 2.06 (dt, $J = 11.8$, 6.1 Hz, 2H). $^{13}$C NMR (100.3 MHz, CD$_3$CN) $\delta$ 161.1, 153.3, 152.8, 144.9, 144.2, 142.7, 142.2, 133.7, 129.1, 129.0, 128.7, 124.3, 119.6, 119.2, 114.0, 112.2, 72.5, 71.3, 71.1, 71.0, 70.9, 70.1, 69.8, 67.3, 58.8, 41.2, 40.9. ESI MS ($m/z$) calculated for [C$_{84}$H$_{108}$N$_6$O$_{19}$H]$^+$ 1505.77, found: 1505.80.
Figure S1. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) spectra of compound 4 in CDCl$_3$. 
Figure S2. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) spectra of compound 5 in CDCl$_3$. 
Figure S3. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) spectra of compound 1 in MeCN-d$_3$. 
Figure S4. $^1$H-NMR (top) and $^{13}$C-NMR (bottom) spectra of compound 2 in MeCN-d$_3$. 
3. Supporting experimental figures

Figure S5. Determination of the photo-stationary state in acetonitrile by $^1$H-NMR. (A) Azobenzenes compound states. Upon UV irradiation the initial trans-trans azobenzene switches to the cis-trans and cis-cis forms. (B) $^1$H-NMR (400 MHz in CD$_3$CN) spectra of chiral compound 1 and (C) achiral compound 2 at the initial state (red spectra) and after UV light irradiation ($\lambda = 365$ nm, green spectra). (D) Ratio of the $^1$H-NMR of the integrated peaks (highlighted by the arrows) corresponding to the trans/cis signals of compounds 1 and 2. The smaller proportion of the cis isomer in the case of compound 1 is probably due to the fact that the photostationary state was not reached at that moment. Irreversible photo-degradation became more prominent and have not allowed us to analysis the NMR spectra unambiguously.
Figure S6. UV-vis spectra of the isolated single building blocks 1 and 2 in acetonitrile. (A) After UV irradiation and relaxation under ambient light for the chiral compound 1. (B) Cycles of UV irradiation (λ = 365 nm, 15 s) and relaxation with the ambient light for the chiral compound 1. (C) UV-vis spectra of thermal relaxation of the chiral azobenzene 1 and (D) achiral azobenzene 2 in dark. (E) and (F) Linear fitting of the relaxation for azobenzenes 1 and 2 taking into account the reversibility of the reaction due to the low intensity of the UV light. The law rate is $v = k_1[\text{cis}] - k_2[\text{trans}]$. $A_\infty$ is the absorption at the initial state at 360 nm and $A_t$ the absorbance at the time $t$ at 360 nm.
Figure S7. TEM micrographs of the tubules. (A) TEM of the tubules with the chiral building block 1 (83 µM). (B) Grey value profile of an area of TEM picture a. (C) TEM of the tubules with the achiral building block 2 (80 µM). (D) Grey value profile of an area of the TEM picture C.

Figure S8. AFM visualization of the tubules. (A) AFM phase image of the synthetic tubules self-assembled from the achiral building block 2 in water and deposited on mica. (B) Height profile from the AFM image.
Figure S9. Measurements of the external diameter from the periodicity of the bundled tubes 2 (1.33 mM) observed by Cryo-TEM. External diameter of the tubules was measured at 3 different areas and subsequently averaged.

<table>
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<td>11.2</td>
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Figure S10. 3D model of the tentative hexameric non-covalent macrocycle composing the tubules with the characteristic diameters (Chem3D®).

Figure S11. Determination of the critical aggregation concentration. Emission spectra of Nile Red solution (0.94 μM) in water with different concentration of (A) chiral azobenzene 1 and (B) achiral azobenzene 2.
Figure S12.Modification of the diameters of the tubules 2 (1.33 mM) before and after 20 minutes of UV light irradiation. The measurements have been done by using the difference of the contrast on pictures at different areas of bundled tubules. Values are given in nm.
Figure S13. Comparison of the number of the tubules 2 (1.33 mM) before and after 60 minutes of UV light irradiation.
Figure S14. Dynamic light scattering measurements of the size of the tubules formed by compound 2 in water before and after UV irradiation. The concentration of the solution was 100 μM. Black line – before UV light irradiation and red line – after UV light irradiation for 100 min.

Figure S15. Estimation of the trans/cis ratio during the UV irradiation process at 35 min. From the calibration curve of the absorption at 360 nm (30520 L.mol⁻¹.cm⁻¹, cis form has negligible absorption at this wavelength) at changing concentration of the compound 1 in water, the amount of the trans form was estimated to be 83 %.
Figure S16. Observation of the 3 step disassembly process of the tubules by UV-vis for the achiral building block 2 in water. (A) UV-vis spectra upon UV irradiation. (B) \( \lambda_{\text{max}} \) versus irradiation time and the 3 observed steps. As observed for the tubule formed by chiral building block 1, a red shift appeared at the initial state showing that the structure of the tubules is changing. In the second step the \( \lambda_{\text{max}} \) remains constant while the tubules break up into shorter fragments. Finally, the fully disassembly is accompanied by the blue shift of the \( \lambda_{\text{max}} \).

Figure S17. Photo-triggered disassembly of tubules formed by compound 1 (2.5 \( \mu \)M) in water with encapsulated Nile Red (0.94 \( \mu \)M). (A) UV-vis, (B) CD and (C) fluorescent spectra upon UV irradiation.
Figure S18. Comparison of the initial state of the disassembly process in pure water and water/acetonitrile mixture (95/5). (A) UV-vis and (B) CD spectra of a solution of the tubules formed by 1 at 2.5 μM in water/acetonitrile mixture (95/5) upon UV irradiation. (C) UV-vis and (D) CD spectra of a solution of tubules formed by 1 at 2.5 μM in water upon UV irradiation. (E) CD signal at 390 nm and (F) UV-vis $\lambda_{max}$ shifts vs irradiation time in water (black dots) and in water/acetonitrile mixture (red dots).
4. Computational methods

Construction of the atomistic models and equilibration AA-MD simulations. The atomistic (AA) models for monomer 1 and supramolecular tubule 1 were built and parametrized according the procedure recently adopted for similar self-assembling motifs (5-9). In particular, the AA model of monomer 1 was parametrized based on the General Amber Force Field, GAFF (gaff.dat) (10). The initial structure of tubule 1 was built starting from eleven hexagonal macrocycles, each containing six monomers 1 (Fig. 1a), which have been stacked on the top of each other along z-direction (initial stacking distance of 4.4 Å) with a mutual tilting angle of 16.4°. Such a starting configuration for tubule is consistent with recent literature report on a similar tubule (11). This tubule portion (composed of 66 monomers) was inserted into a simulation box (initial $X \times Y \times Z$ dimensions of $12 \times 12 \times 4.84$ nm) grazing the tube in z-direction and filled of explicit TIP3P (12) water molecules. In this way, replicated along z via periodic boundary conditions, this system effectively modeled a portion of the bulk of an infinite (helical) tubule 1.

All simulations were conducted with the GROMACS 5.1.2 software (13). After initial minimization, tubule 1 was equilibrated for 150 ns of AA-MD in periodic boundary NPT (constant N: number of atoms, P: pressure and T: temperature) using a timestep of 2 fs and a 10 Å cutoff. This simulation time was sufficient for the tubule to reach stable structural and energetic equilibration in the AA-MD regime (Fig. S15). All AA-MD runs were conducted at 300 K (27°C) using the v-rescale (14) thermostat (coupling constant of 2.0 ps) and 1 atm of pressure using semi-isotropic pressure scaling (compatibly with the directional nature of the tubule) with coupling constant of 2 ps. The particle mesh Ewald (PME)(15) approach was used to treat long-range electrostatics. The LINCS algorithm was used to constrain all bonds involving hydrogens (16).

Well-tempered metadynamics simulations. The WT-MetaD (17) simulations have been conducted using the GROMACS 5.1.2 software (13) and the PLUMED 2 plugin (18). We used the C-N=N-C dihedral angle in the azobenzene tail of monomer 1 as the collective variable (CV) describing/biasing the trans-cis transition. In the WT-MetaD runs, we used a HILLS height of 0.28 kcal mol$^{-1}$ and Gaussian SIGMA of 0.35 rad. A bias factor of 10 or 40 was used for the WT-MetaD simulations of the trans-cis transition in monomer 1 as respectively disassembled (in water) or assembled in the tubule. The other simulation parameters are the same reported above. From the WT-MetaD simulations we obtained the free-energy profiles for the trans-cis transition of the azobenzene groups in monomer 1 as disassembled in water or assembled in the tubule (Fig. S16a: black and red respectively). The transition requires more energy in the assembled state compared to the disassembled one due to the crowding present in the assembly (at least $\sim$20 kcal mol$^{-1}$ for an assembled monomer vs. $\sim$9 kcal mol$^{-1}$ for a disassembled one). In the experiments, light irradiation provides the energy necessary to this transition. We used this information to set up out-of-equilibrium AA-MD simulations where trans-cis transitions in the tubule could be monitored iteratively.

Out-of-equilibrium AA-MD simulations and analysis of strain build-up. We modified the native dihedral angle potential of the azobenzene groups in monomer 1 to observe spontaneous trans-cis transitions during an AA-MD run (Fig. S16b). This is equivalent to adding a static bias to the azobenzene dihedral potential of the monomers in tubule 1. In the equilibrium AA-MD, the C-N=N-C dihedral potential of the native azobenzene groups in monomer 1 was defined by the sum of two terms as in Eq. S1 (standard for this group in the GAFF force field):
\[ V_{d,\text{native}}(\phi) = k_1 \left(1 + \cos(n_1 \phi - \phi_{1,s})\right) + k_2 \left(1 + \cos(n_2 \phi - \phi_{2,s})\right) \]  
\[ (S1) \]

where \( k_1 = 3 \text{ kcal mol}^{-1} \), \( n_1 = 2 \), \( \phi_{1,s} = 180^\circ \) and \( k_2 = 2.8 \text{ kcal mol}^{-1} \), \( n_2 = 1 \), \( \phi_{2,s} = 0^\circ \) (Fig. S16b: black curve). In the out-of-equilibrium AA-MD runs, such dihedral potential was modified as in Eq. S2 for all monomers in tubule 1:

\[ V_{d,\text{out-of-equlil}}(\phi) = k_3 \left(1 + \cos(n_3 \phi - \phi_{3,s})\right) \]  
\[ (S2) \]

where \( k_3 = 6.2 \text{ kcal mol}^{-1} \), \( n_3 = 1 \), \( \phi_{3,s} = 180^\circ \) (Fig. S16b: red curve). This moved the \textit{trans} configuration of the monomers in pre-equilibrated tubule 1 out-of-equilibrium, favoring transition to \textit{cis} inside the tubule. The parameters of Eq. S2 were optimized to obtain a bias on the azobenzene dihedral potential consistent with the energy necessary for the \textit{trans-cis} transition in an assembled monomer captured by WT-MetaD (~20 kcal mol\(^{-1}\)). This was verified to be the minimum bias to guarantee sufficient speed-up to allow us monitoring the \textit{trans-cis} transitions in the timescale of the AA-MD run.

All other simulation parameters in these AA-MD runs were the same used for the equilibration runs (see above). During these out-of-equilibrium AA-MD simulations we monitored the \textit{trans-cis} transitions of the azobenzene groups in the tubule using \texttt{gmx_angle}. The average energy absorbed by each assembled monomer in the tubule as a function of the percentage of \textit{trans-cis} transitions (Fig. 7b: strain build-up) was calculated as: \( \Delta E = E_{\text{perturbed}} - E_{\text{native}} \), where \( E_{\text{native}} \) is the average energy of the monomers in the unperturbed equilibrated tubule 1, while \( E_{\text{perturbed}} \) is the average energy of the (modified) monomers during the out-of-equilibrium AA-MD (\( \Delta E > 0 \) identifies unfavorable energy variation/accumulation). The \( \Delta \Phi \) parameter (Fig. 7c: defined as \( \Delta \Phi = \Phi_{\text{perturbed}} - \Phi_{\text{native}} \)) measures the deviation during the runs from the parallel orientation of the planes defined by the aromatic rings in the monomers in equilibrated tubule 1 (stacking destabilization). The \( \Phi \) parameter was calculated using the PLUMED plugin. \( \Delta \Phi = 0 \) means that the initial stacked configuration is perfectly preserved during the run, while the higher the \( \Delta \Phi \), the higher the structural distortions in the assembly. All reported data were calculated as the average of three out-of-equilibrium runs.
5. Additional data from AA-MD simulations

Figure S19. AA-MD equilibration of tubule 1. (A) Energy of tubule 1 as a function of AA-MD simulation time. (B) Root mean square deviation (Rmsd) of the atoms of tubule 1 as a function of AA-MD simulation time. (C) Deviation (in percentage) in the order parameter ($\Delta \Phi$) as a function of simulation time calculated respect to the ordered assembly of the monomers in equilibrated tubule 1. All data show that tubule 1 reaches stable equilibration along the AA-MD run.

Figure S20. Azobenzene trans-cis transition in monomer 1. (A) Free-energy profiles for trans-cis transition as captured by WT-MetaD simulations in disassembled in monomer 1 (black) or in monomer 1 as assembled in the tubule (red). (B) Dihedral potential term for the azobenzene trans-cis transition in monomer 1. Black: original non-modified dihedral (GAFF force field, Eq. S1). Red: modified dihedral (see Eq. S2) used in the out-of-equilibrium AA-MD simulations.
5. References


